

**The Effect of Prebiotic and Probiotic Supplementation on Intestinal Maturity in Turkey
Poults**

Honors Research Thesis

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ABSTRACT

The turkey industry operates by utilizing the least feed possible to achieve the highest quality muscle products attainable. Currently, feed grade antibiotics are used to increase the feed conversion ratio, or how much feed is required per pound of gain. However, antibiotics have become less attractive to consumers and several countries have banned them in meat production completely due to the potential for creating antibiotic resistant bacteria. Two potential alternatives to enhance growth are probiotics and prebiotics. The purpose of this study was to determine the growth stimulating potential of two probiotics, *Bacillus subtilis* and *Bacillus licheniformis* and one prebiotic, a mannanoligosaccharide (MOS). Four groups of turkey poults were fed a commercial diet and three were supplemented with one of the feed additives. Turkeys were euthanized at 11 days post hatch and samples of the small intestine were collected to assess intestinal maturity. Morphological parameters were examined to determine if any of the treatments accelerated intestinal maturity. MOS and *Bacillus licheniformis* supplementation increased acidic goblet cell density. *Bacillus subtilis* had a negative impact on many intestinal parameters while exerting a positive effect on goblet cell density.

BODY

Introduction

Poultry producers are always looking for new methods to increase production. It is the industry standard to use food grade antibiotics to promote growth. However, constant use of these drugs can potentially lead to antibiotic resistant strains of bacteria. There are other possible options for maintaining high levels of production which come at a lower societal price.

Probiotics and prebiotics have been shown to increase the efficiency of growth, however more research is required to ascertain their efficacy, proper dosage and cost-effectiveness (Smirnov et al., 2004; Solis de los Santos et al., 2007; Tsirtsikos et al., 2012).

An important parameter in the efficiency of growth is intestinal maturity. A mature intestine is more adept at converting feed into energy which can be measured by the feed conversion ratio. Antibiotics increase the ratio by preventing illness which would otherwise slow growth and divert caloric resources to fight infection (Smirnov et al., 2005). If a dietary supplement such as a probiotic or prebiotic can achieve similar results without the potential side effect of bacterial resistance, antibiotics may become unnecessary except in the case of illness.

The health of the gastrointestinal tract can be measured not only by the length and area of absorptive villi, but also by the number and type of goblet cells, a specialized epithelial cell. Goblet cells are responsible for producing mucins, high molecular weight glycoproteins that serve to anchor commensal bacteria and exclude pathogenic species (Specian and Oliver, 1991). Logically, the density of goblet cells should correspond to the thickness of the mucous layer, an aqueous substance composed of immunoglobulins, dead epithelial cells, electrolytes and mucin glycoproteins (Specian and Oliver, 1991). Thus, increasing goblet cell density thickens this protective layer. Mucins can be divided into two main categories: neutral and acidic. Acidic

mucin is more resistant to pathogenic bacteria attempting to digest its constituent carbohydrate side chains (Forder et al, 2007). A gut with a higher proportion of acidic mucins is better protected from infection and the animal can focus its resources on growth.

Probiotics are one option for dietary supplementation which can increase intestinal maturity. Probiotics are commensal species of bacteria that will ideally colonize the intestine and prevent harmful species from attaching through a process called “competitive exclusion” (Smirnov et al., 2005). Smirnov et al. (2005) found that probiotic supplementation in commercial broiler chicks resulted in a similar goblet cell density to control chicks, but a larger villus area and goblet cell size. In a study by Tsirtsikos et al. (2012), broiler chicks supplemented with probiotics exhibited increased cecal villus height and crypt depth.

A second option is a prebiotic such as a mannan-oligosaccharide (MOS). This unique class of compounds, composed of both protein and carbohydrates, binds to certain types of pathogens such as *E. coli* and prevents them from attaching to the wall of the small intestine. Bacteria cannot break down MOS and are flushed from the system. Additionally, the carbohydrate fraction may serve as a substrate for beneficial bacteria which are necessary for a healthy digestive system (Solis de los Santos et al., 2007). With reduced challenges from pathogens in prebiotic supplemented birds, goblet cells should proliferate and villus surface area should increase more quickly than the non-supplemented group.

One commercial MOS is Alphamune, a yeast extract that contains a mannan-oligosaccharide. Solis de los Santos et al. (2007) found that supplementation with Alphamune resulted in increased villus height, villus area, crypt depth and density of both acidic and neutral mucin producing goblet cells compared to the control. Additional supportive research will encourage turkey producers to utilize these alternative methods of growth promotion instead of

food grade antibiotics. Perhaps the correct dose of prebiotics and probiotics together can achieve a similar effect on growth and the feed conversion ratio.

This project tested two commercial probiotics, *Bacillus subtilis* and *Bacillus licheniformis*, and one prebiotic, a mannan-oligosaccharide isolated from *Saccharomyces cerevisiae*, against a control to determine if any of the dietary supplements can accelerate maturation of the intestine.

Materials and Methods

Turkeys were raised at the Ohio State University Wooster Poultry Research Center and fed a commercial diet which met NRC (1994) requirements. There were four groups to which 20 poultts were assigned using a completely randomized design. Poultts in each of three treatment groups were fed a recommended commercial dose of one of three supplements: Probiotic A - *Bacillus subtilis*, mannan-oligosaccharide, or Probiotic B - *Bacillus licheniformis*. Poultts were euthanized at eleven days post hatch and tissue samples were collected by Stephanie Loeffler for her graduate project. A 1 inch section of the lower small intestine was taken between Meckle's Diverticulum and the ileo-cecal junction and stored in Prefer fixative (Anatech Ltd., Battle Creek, MI). Sections were stained with eosin and Periodic Acid-Schiff by the Goss Laboratory in the Ohio State University College of Veterinary Medicine. These slides were used to collect data on villus morphology. Sections were stained with a combination of periodic acid-Schiff and Alcian Blue pH 2.5 in house in order to stain the goblet cells for both neutral and acidic mucins (Forder et al., 2007). Photographs of ten goblet cells per bird were taken with a microscope mounted digital camera. Morphological measurements and goblet cell counts were collected using ImageJ software (NIH Bethesda, MD). Density numbers were calculated from these

measurements. Statistical analysis was performed using ProcGLM in SAS. Duncan's multiple mean range tests was utilized for mean separation.

Results

Average villus height did not vary between treatments. Villus area and crypt depth were not significantly different in MOS or Probiotic B treatments. However, the Probiotic A group had both a reduced villus area and decreased crypt depth compared to the control (Table 1).

Diet	Villus Ht (μm)	Villus Area ($\mu\text{m}^2 \times 10^4$)	Crypt Depth (μm)	Acidic Cells (no.)	Cells/Height (no./ μm)	Cells/Area (no. $\times 10^3/\mu\text{m}^2$)
Control	1038	28.6617 ^a	205 ^a	42.4 ^c	.041 ^b	.151 ^c
Probiotic A	949	20.8854 ^c	175 ^b	46.8 ^b	.050 ^a	.237 ^a
MOS	1019	26.9796 ^{ab}	211 ^a	50.5 ^a	.051 ^a	.202 ^b
Probiotic B	1021	25.6081 ^{ab}	205 ^a	52.1 ^a	.052 ^a	.216 ^b
Pooled SEM	6	.4772	2	0.6	0.001	0.005

Table 1: Differences in cellular morphology of an average villus between the four experimental groups represented as least square means. Superscripts represent Duncan's multiple mean separation. Means with different superscripts within the same column differ significantly with a threshold of $P \leq 0.001$.

All treatments showed an increase in average acidic goblet cell number per villus when compared with the control. The MOS and Probiotic B treatments were the most significant (Figure 1). All three treatment groups increased acidic goblet cell density in terms of villus area and height, with Probiotic A showing the largest increase over the control in terms of villus area (Table 1).

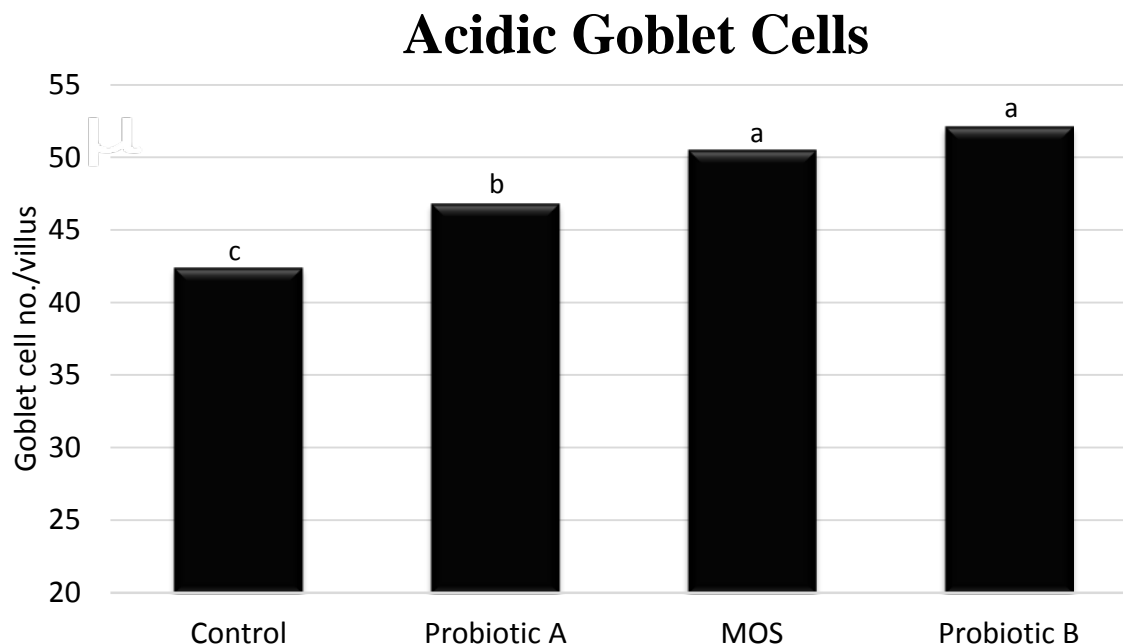


Figure 1: Effect of treatments on acidic goblet cell count per villus as measured by an average of 10 villi per bird.

Discussion

The intestinal mucous layer is the first line defense against ingested pathogenic bacteria. It serves as a physical barrier preventing toxins and enteric bacteria from reaching the epithelium and by extension, the bloodstream (Specian and Oliver, 1991). The composition of the intestinal mucous layer is vital in the young bird as infections such as necrotic gastroenteritis are responsible for slow growth and economic loss for the producer. Higher levels of acidic mucins are associated with a more mature, protected intestine (Forder et al., 2007).

MOS supplementation had a positive effect on acidic goblet cell density. This result is consistent with a study by Solis de los Santos et al. (2007). However that study also found an increase in other morphological parameters such as crypt depth. Probiotic B, *Bacillus licheniformis*, similarly increased the density of acidic goblet cells but had no statistical effect on other measurements of intestinal morphology. These results align partially with *B. licheniformis*

research by Dong et al. (2012) which found no change in either acidic mucin production or villi measurements of 14 day old broiler chicks.

It is intriguing that supplementation with Probiotic A had a negative effect on both villus area and crypt depth. It is possible that this was caused by a small sample size and the wide variation in intestinal morphology seen in young poults. This observation stands in stark contrast to a study by Thangavel et al. (2013) who reported an increase in villus height and crypt depth in broiler chickens supplemented with *Bacillus subtilis* when challenged with *Clostridium perfringens*. Another possible explanation for the discrepancy is that the positive effects of this probiotic are much more pronounced when the host is actively fighting a pathogenic attack.

The lack of a positive impact of Probiotic A compared with Probiotic B emphasizes the differences that exist in commercial products that utilize the same genus of bacteria, albeit different species. This variability of impact makes turkey producers less likely to trust and utilize these potentially beneficial microbes. However, in this study and many others, probiotic B and the prebiotic MOS were found to have a positive impact on the intestinal morphology of young turkey poults. This data supports the argument in favor of their supplementation.

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